The whole rock oxygen isotopic composition ($\delta^{18}O$) of the fossil bed varies from +11.83 to +12.13 per mil (*6*) along the outcrop length of the bed. Comparison of these values with those measured on unaltered or silicified Devonian brachiopods (*7*) shows that the fossils have been depleted by 12 per mil as a consequence of metamorphism.

The correlation by Billings (*1*) of the rocks of the Mount Moosilauke septum with the Littleton Formation was based, in part, on the identification of a brachiopod collected 5 km west of the Beaver Brook locality (*8*). The phyletic identification of the form has been questioned, however (*9*). The discovery of the Beaver Brook fossils and their stratigraphic age assignment completely confirms Billings' original correlation. The probable Oriskany age of the Beaver Brook fossils suggests that much of the Littleton Formation may be older than the Schoharie age rocks of the type area (*9*). The older age of the rocks in the Mount Moosilauke septum is compatible with what is known of lithostratigraphy in the Devonian of New England: the rhythmically banded and laminated clastic beds prevalent in the Mount Moosilauke septum are similar in appearance to those of the Oriskany age Sebomook Formation of northern Maine (*10*).

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Fallopian Tube Isthmic Mucus and Ovum Transport

Abstract. *The oviduct isthmus is capable of transporting spermatozoa and ova in opposite directions. A column of tenacious mucus that occupies the lumen of the rabbit oviduct isthmus during estrus may permit sperm transport. After ovulation the mucus disappears, with subsequent efflorescence of cilia, which probably assist transport of ova to the uterus.*

Ovulation in the estrous rabbit normally occurs between 9.5 and 14 hours after coitus or the injection of exogenous luteinizing hormone (*1*). Ova reach the ampullary-isthmic junction (AIJ) less than 10 minutes after being released (*2*). Paralysis of the ampullary musculature with a β-receptor agonist has no effect on ampullary transit time (*3*), implying that cilia provide the most important propellant force. Spermatozoa, having begun to reach the ampulla well before ovulation, are present in large numbers by 10 hours after coitus (*4*). Fertilization normally takes place in the ampulla.

Whether or not fertilization has occurred, the ovum or zygote is delayed at the AIJ for 24 to 36 hours before entering the isthmus (*5*). The tubal isthmus, located between the site of fertilization and the site of implantation, needs the unique capability of effecting sequentially the transport of spermatozoa and ova in opposite directions. Progression of the egg along the isthmus is gradual and, although there is no evidence for any specific delay at the uterotubal junction, ova do not normally arrive in the uterus until 66 to 72 hours after ovulation (*5, 6*).

High doses of exogenous estradiol can prolong the delay at the AIJ (*5*), a process known as tube locking (*7*). In contrast, administration of progesterone 24 to 48 hours before ovulation accelerates ovum transport by reducing both the delay at the AIJ and isthmic transit time (*5*). The physiological basis for the isthmic delay is not known (*6, 8*). Adrenergic nerves supply the thick muscular wall of the isthmus (*9*) and it seems that both stimulatory (α) and inhibitory (β) adrenergic receptors are present (*10*). Exogenous estrogen increases the noradrenalin content in the oviduct, and, conversely, the tube-locking effect of estradiol may be overcome by the concomitant administration of an α-receptor blocking agent and progesterone (*11*). However, destruction of adrenergic nerves with 6-hydroxydopamine, depletion of noradrenalin with reserpine, or anatomical denervation have no deleterious effect on the reproductive capacity of the rabbit (*10*). It has therefore been concluded that the muscular activity of the oviduct has a minimal influence on isthmic ovum transport (*7, 10*).

Segments of oviduct isthmus were removed from eight normal adult New Zealand White rabbits and from five rabbits in which ovulation had been induced with human chorionic gonadotrophin (hCG). These segments were prepared for scanning electron microscopy by fixation with 3 percent glutaraldehyde, dehydration in acetone, critical-point drying with CO_2, and shadow-casting with gold. The appearance of ampullary and fimbrial mucosa from these rabbits is generally similar to that of the human fallopian tube (*12*), except for a greater predominance of ciliated cells in the rabbit, and cilia always appear prominent and erect.

When care was taken not to disturb the mucosal surface by, for example, excessive washing, oblique sections through the oviduct isthmus of the estrous rabbits revealed the presence of dense and tenacious mucus (Fig. 1A). This mucus typically obliterates the tubal lumen, but in areas in which it is absent secretory cells are conspicuous; they have tall distended apices bearing prominent microvilli, and often demonstrate apocrine secretory activity (Fig. 1B). The ciliated cells are indistinct; cilia droop randomly and may be caught in the strands of mucus.

Twenty-four hours after ovulation was induced with hCG (100 units, intravenously), much of the viscous mucus had disappeared. Previously agglutinated cilia were prominent and erect, and the apices of the secretory cells appeared sunken in comparison. In three rabbits in which progesterone was administered (2.5 mg in oil, intramuscularly) 24 hours before the hCG injections, a maneuver that accelerates isthmic ovum transport (*5*), the cilia were even more prominent, and ciliated cells dominated the isthmic mucosa (Fig. 1C). This situation was similar to that seen 48 and 72 hours after injection of hCG alone. In contrast, the isthmic mucosa of three rabbits injected simultaneously with estradiol cyclopentylpropionate (250 μg, intramuscularly) and hCG, a regime that causes tube locking (*5*), showed persistent secretory cell dominance and ciliary depression.

In 1958, Greenwald (*13*) demonstrated the prominence of rabbit isthmic ciliated cells 3 days after coitus by conventional

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histological techniques; this observation is consistent with our findings. Koester (*14*) confirmed these observations and, using radioactive sulfur and autoradiography, found that isthmic secretion suddenly increased after ovulation and then decreased as the corpora lutea formed. He attributed these changes to estrogen and progesterone, respectively.

The isthmic mucosa of the untreated rabbit closely resembles the mucus-laden columnar epithelium of the human endocervix (*12*). In both cases the mucus has a granular, filamentous appearance, similar to that of aspirated human cervical mucus observed with scanning electron microscopy after critical-point drying (*15*). While this method of dehydration may be excellent for solid tissues, it probably introduces considerable artifactual distortion to the delicate glycoprotein base of viscous-phase mucus. Daunter and colleagues (*16*), using fixation in 0.6 percent glutaraldehyde and slow freeze-drying, have demonstrated that human cervical mucus consists of an interconnecting system of thin membranes, giving a honeycomb structure with no free filaments; at midcycle spermatozoa were seen within the channels of the honeycomb. When this technique was applied to the isthmic mucus of the rabbit oviduct, a similar membranous honeycomb structure was seen (Fig. 1D).

In rabbits, both the cervix and the tubal isthmus reduce the numbers of sperm recoverable distally, and the fertilizing power of the sperm that are able to pene-

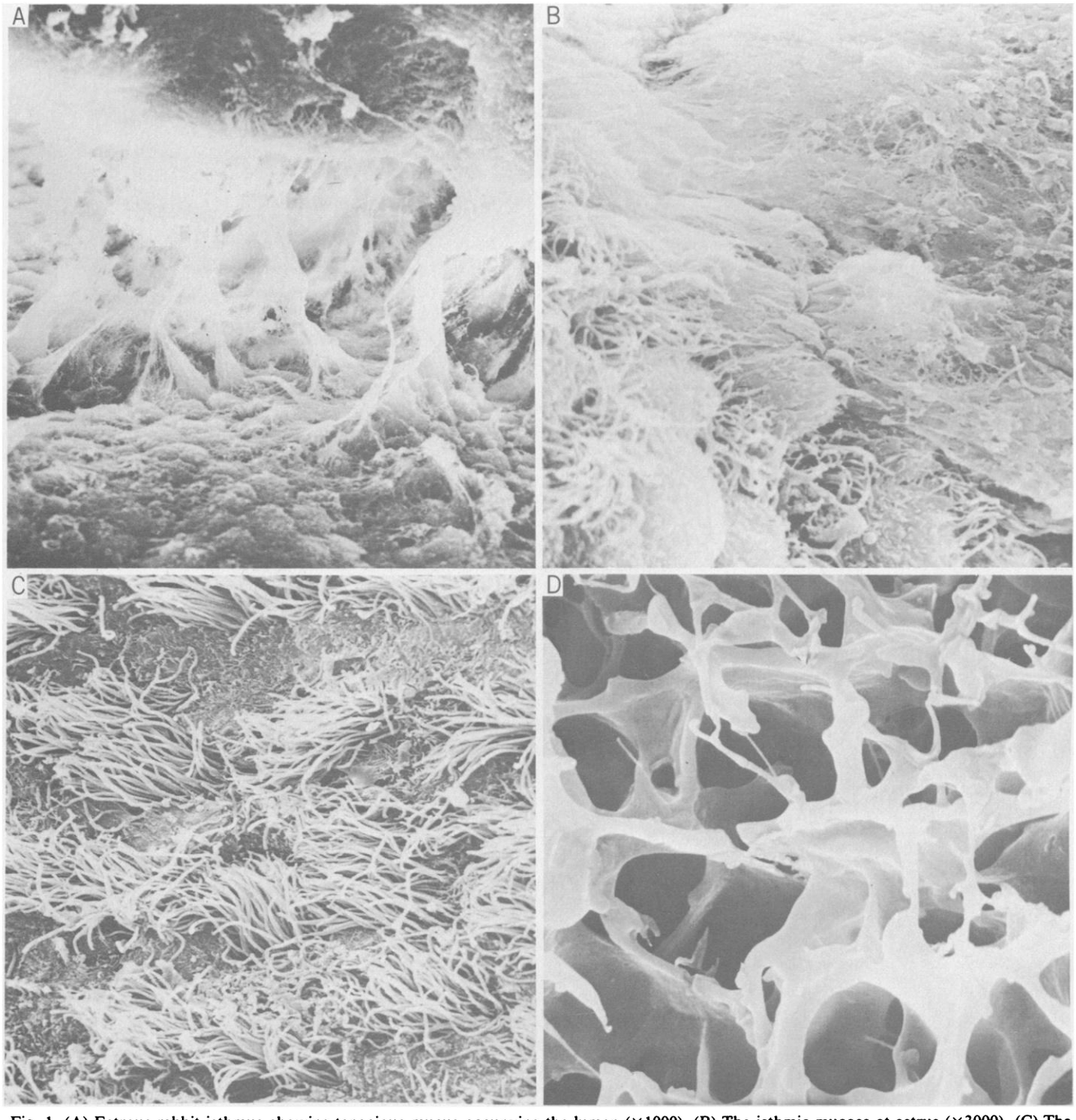

Fig. 1. (A) Estrous rabbit isthmus showing tenacious mucus occupying the lumen (×1000). (B) The isthmic mucosa at estrus (×3000). (C) The isthmic mucosa 48 hours after progesterone and 24 hours after hCG (×3000). (D) Estrous rabbit isthmic mucus fixed with 0.6 percent glutaraldehyde and freeze-dried at 0.1 torr for 12 hours, showing honeycomb structure (×1000).

trate each region is enhanced sequentially (*17*). Further evidence for sperm selection in the oviduct comes from the observation that the motility of sperm recovered from the isthmus is poorer than that of sperm that have reached the ampulla (*4*). Both the cervix and the fallopian tube in humans contain large amounts of β-amylase (*18*), an enzyme that in the rabbit both aids capacitation of spermatozoa in vitro and causes antigenic changes similar to those found in sperm recovered from the ampulla (*19*). No explanation has been advanced for the sperm filtering and possible capacitating functions of the tubal isthmus, although the same functions in the cervix are attributed to its mucus.

These observations indicate that the ability of the oviductal isthmus to transport, bidirectionally, sperm and ova may depend, in part, on the presence or absence of a column of viscous mucus. Before ovulation the transport of sperm distally appears to be independent of cilial activity, and the mucus may serve a role in sperm selection similar to that proposed for the cervix. Within several days after ovulation the mucus disappears, allowing cilia to become erect and, presumably, able then to function efficiently in transporting the egg to the uterine cavity.

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Red Cell Membrane Glycophorin Labeling from Within the Lipid Bilayer

Abstract. *Human red blood cell membranes were labeled from within the lipid bilayer by the apolar photosensitive reagent, 5- [^{125}I]iodonaphthyl-1-azide. Glycophorin, the major sialoglycoprotein of the red cell membrane, was purified by two different methods; it contained approximately half of the total label incorporated into membrane proteins. The label was confined to the trypsin-insoluble peptide of glycophorin that includes a sequence of 20, mainly apolar, amino acids. These findings provide direct evidence that the labeled segment resides within the membrane in direct contact with the lipid bilayer, and support the suggestion that glycophorin spans the bilayer through its hydrophobic domain.*

Proteins in biological membranes are thought to have segments of their polypeptide chains in contact with or spanning the phospholipid bilayer (*1, 2*). It is likely that these domains contain specific sequences of amino acids that structurally and functionally distinguish membrane proteins from other soluble cellular proteins. The isolation and characterization of lipid-embedded polypeptides have been curtailed by the lack of direct methods to identify them. Only in the case of glycophorin, the major glycoprotein of the human red blood cell (RBC), does evidence exist for its configuration within the lipid bilayer. Glycophorin has been labeled from both surfaces of the membrane, isolated with water-soluble reagents, and digested with trypsin; the water-soluble, labeled peptides that were released could be identified as those residing in contact with the aqueous environment, while the unlabeled, indigestible peptide was suggested to be that region of the polypeptide chain buried within the bilayer (*3, 4*). Although this experimental approach was valid for glycophorin, a relatively short (131 amino acids) linear polypeptide chain, it could lead to erroneous results if significant regions of the polypeptide chain (or chains) in the aqueous environments were not labeled.

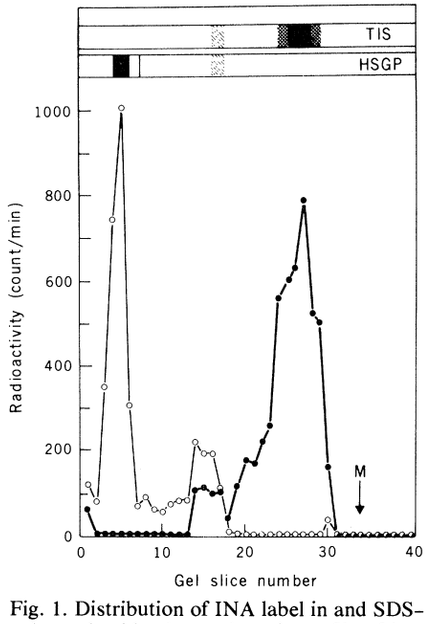

Fig. 1. Distribution of INA label in and SDS-polyacrylamide electrophoretic profile of isolated glycophorin (human sialoglycoprotein, *HSGP*) and its insoluble peptide *(TIS)* after trypsin digestion. Membranes were labeled with INA as described in the text and in (*6*), and glycophorin was isolated by affinity chromatography (Table 1) (*7*). A portion of labeled glycophorin was added to 200 μg of unlabeled glycophorin and trypsinized, and the insoluble peptide *(TIS)* was isolated (*3*). Intact labeled glycophorin (○) and *TIS* (●) were subjected to electrophoresis on 12 percent acrylamide gels in the presence of 1 percent SDS (*3*). The gels were stained for protein with Coomassie brilliant blue and cut into 2-mm slices, and the radioactivity was counted; *M* denotes the transfer dye.

A direct procedure for labeling those portions of membrane proteins in contact with the bilayer lipids had been recently developed (*5, 6*). The very hydrophobic, light-sensitive compound 5-[^{125}I]iodonaphthyl-1-azide (INA), is nonreactive in the dark, and more than 98 percent of it partitions into the lipid phase of the membrane (*6*). Subsequent short irradiation at 314 nm converts INA into a reactive nitrene that attaches covalently to integral proteins and lipids of the membrane from within the bilayer (*5, 6*).

We have labeled RBC membranes with INA and, after isolating the labeled glycophorin, have determined the distribution of the label within the polypeptide chain. Our results indicate that the trypsin-insoluble segment (TIS) contains nearly all the incorporated label. The results further provide direct evidence that TIS is located within the bilayer, and confirm the usefulness of INA in identifying polypeptides embedded in the lipid phase.

Ghosts from RBC were prepared from freshly drawn blood and were labeled with INA (*6*). To 2.0 ml of the ghost suspension (1 mg of protein per milliliter) in phosphate-buffered saline were added 20 μl of an ethanol solution of [^{125}I]INA (1.3 Ci/mmole), producing a final concentra-

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